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Original Article

Genetic characterization of *Haemonchus contortus* from slaughtered goats in Cha-am District, Phetchaburi Province, Thailand

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Abstract

Haemonchus contortus is a gastro-intestinal parasite. The parasites were collected from the abomasum of 30 slaughtered goats from a local slaughterhouse in Cha-am District, Phetchaburi Province, Thailand. Co-infection of *H. contortus* and *H. placei* was found in 24 goats. The prevalence of *H. contortus* was 80% while *H. placei* was 86.67% . This study determined the genetic variation using 20 *H. contortus* samples for amplification and sequencing that used the internal-transcribed spacer-2 (ITS-2) and nicotine amide dehydrogenase subunit 4 (*nd4*) gene. The nucleotide sequence analysis revealed 9 genotypes (ITS-2) and 19 haplotypes (*nd4*) among 20 and 19 samples, respectively. Phylogenetic tree analysis using both ITS-2 and *nd4* sequences showed a close relationship within the *H. contortus* population in this area. Since haemonchosis in goats is still a serious problem which causes economic losses, knowledge on the genetic variation of *H. contortus* in Thailand may have implications for epidemiology and further parasite control programs.

Keywords: *Haemonchus contortus*, ITS-2, *nd4*, genetic variation, Thailand

1. Introduction

Haemonchus contortus (barber's pole worm) is a trichostrongyloid nematode parasite which causes haemonchosis in small ruminants such as sheep and goats in tropical and subtropical countries in the world (Emery, Hunt, & Le Jambre, 2016; Jacquiet, Cabaret, Thiam, & Cheikh, 1998; O'Connor, Walkden-Brown, & Kahn, 2006) and causes significant economic losses worldwide (Achi *et al.*, 2003; Hoste, Chartier, & Le Frileux, 2002; Peter & Chandrawathani, 2005). These adult parasites live in the abomasum of the infected host and consume blood which causes body weight loss, paleness, anemia, edema, diarrhea, and even death (Gasser, Bott, Chilton, Hunt, & Beveridge, 2008; Holmes, 1993). The morphology of the adult parasites are different in the female (18-30 mm) which has a longer shape than the male (10-20 mm) and the "barber pole" appearance of white ovaries and uteri that twist around the body for the length of the female worm around the blood-filled intestine while the male shows large copulatory bursa that contains an asymmetrical dorsal lobe and a Y-shaped dorsal ray (Tak *et al.*, 2014). *Haemonchus* females can produce up to 10,000 eggs per day which causes rapid contamination of pastures (Holmes, 1987). Although the primary treatment and control of a *H. contortus* infection has been veterinary anthelmintic drugs for decades, the effectiveness of a cure and the resistant to these anthelmintic drugs have been investigated (Gilleard, 2013). The strategies to control a *Haemonchus* infection were proposed that included development of a vaccine, bioactive forages, pasture contamination management, immune-nutrition, breeding and selection, therapeutic minerals and vitamins or even the development of new drugs (Kearney, Murray, Hoy, Hohenhaus, & Kotze, 2016). In this situation, genetic characterization of *H. contortus* would seem to be not only important in the determination of its genetic structure in several areas and host distributions (Achi *et al.*, 2003; Cerutti *et al.*, 2010) but also the genetic information may provide knowledge on the development of a vaccine, drug target identification, and the mechanism of anthelmintic resistance

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(Akkari *et al.*, 2013; Redman *et al.*, 2008). The genetic variation and characterization of *H. contortus* in goats were reported in several countries (Akkari *et al.*, 2013; Gharamah, Azizah, & Rahman, 2012; Hussain *et al.*, 2014; Yin *et al.*, 2013) including Thailand (Mangkit *et al.*, 2014). The intra-specific genetic variation of the second transcribed spacer (ITS-2) sequence from *H. contortus* was studied in different locations in Thailand (Chiang Mai, Mae Hong Son, Krabi, Prachuap Khiri Khan, Buriram, Chaiyaphum, Suphanburi, Phra Nakhon Si Ayutthaya, Saraburi, Kanchanaburi, and Ratchaburi) and revealed 21 genotypes (Mangkit *et al.*, 2014)

However, the genetic information of *H. contortus* from infected goats in other provinces in Thailand is necessary to investigate the genetic variation of these parasites for further parasite control management. The second internal transcribed spacer (ITS-2) of the nuclear ribosomal DNA sequences are commonly used as markers for species separation among nematodes because the ITS-2 regions are one of the most variable nuclear loci with a high evolution rate (Powers *et al.*, 1997). For *Haemonchus* spp., the ITS-2 sequences were applied in the identification of two species within the genus *Haemonchus* (Gasser *et al.*, 2008) and the intraspecific variation of ITS-2 within *H. contortus* (Steven son, Chilton, & Gasser, 1995). In the evolutionary studies of animal populations of the mitochondrial DNA (mtDNA), the nicotine amide dehydrogenase subunit 4 (*nd4*) and cyto-chrome oxidases (*co*) gene were determined to be useful molecular markers. The mtDNA has a high rate of substitution that facilitates the differentiation between closely related individuals (Blouin, 2002; McDonnell, Love, Tait, Lichten fels, & Matthews, 2000). The *nd4* gene was suggested to be better than the *co* gene because the *co* gene appeared to have strong conservation of amino acid which may limit most of the useful variations to silent sites (McDonnell *et al.*, 2000). Therefore, the previous population studies of *H. contortus* selected the *nd4* gene for genetic variation observation (Cerutti *et al.*, 2010; Troell, Engstrom, Morrison, Mattsson, & Hoglund, 2006).

The ITS-2 sequence was used to differentiate the species of *Haemonchus* genus (Akkari *et al.*, 2013; Gharamah *et al.*, 2012; Hussain *et al.*, 2014; Mangkit *et al.*, 2014; Yin *et al.*, 2013) and the nicotine amide dehydrogenase subunit 4 (*nd4*) gene was used to investigate the population genetic structure (Gharamah *et al.*, 2012; Hussain *et al.*, 2014; Yin *et al.*, 2013). Therefore, this research aimed to study the genetic variation of ITS-2 and *nd4* sequences in *H. contortus* obtained from slaughterhouse goats in Cha-am District, Phetchaburi Province, Thailand and determine the relationships among the ITS-2 and *nd4* sequences of *H. contortus* which was esta-blished in the GenBank database. This present data are able to increase the genetic information of this parasite in Thailand and may provide knowledge for the control of *H. contortus* infection.

2. Materials and Methods

2.1 Study area and worm collection

Adult worms were collected from the abomasum of 30 slaughtered goats at a local slaughterhouse located in Cha-am District, Phetchaburi Province. In this area, the local goat farmers raise goats at areas around their houses and send the goats to a slaughterhouse for family consumption. Therefore, the number of goats in this experiment is quite low. Also, time limitations for this research precluded a larger population of goats. The amounts and types of worm infestation in each of the sampled abomasum were recorded. Multiple worm samples from individual hosts were washed in 0.85% physiological saline and preserved in 70% ethanol. The species and sex of the worms were identified under a stereo microscope (Jacquiet, Cabaret, Cheikh, & Thiam, 1997) and kept at –20 °C until use. Five adult female worms of *H. contortus* were randomly selected and pooled together in each individual host. In this study, only female worms were used in order to clearly differentiate the species between *H. contortus* and *H. placei*. Moreover, it was difficult to obtain adult male *Haemonchus* spp. in this experimental area.

2.2 DNA extraction, PCR amplification and sequencing

2.2.1 DNA extraction

The female parasites were washed one time in 500 µL of sterile purified water (Calbiochem, USA). Total genomic DNA was extracted from the whole parasite using a Genomic DNA mini kit (Tissue) (Geneaid Biotech Ltd., Taiwan) according to the manufacturer's protocol. The extracted DNA was eluted in low salt elution buffer and stored at –20 °C prior to use.

2.2.2 PCR amplification and sequencing

The ITS-2 and *nd4* sequence were amplified using polymerase chain reaction (PCR) from total purified genomic DNA of the worms. The 321 bp of ITS-2 PCR product was produced from the oligonucleotide primers: NC1F (5'-ACGTCTGGTTCAGGGTTGTT-3') and NC1R (5'-TTAG TTTCTTTTCCTCCGCT-3') (Stevenson *et al.*, 1995). For *nd*4 gene amplification, the oligonucleotide primers: NAD4-F (5'-GGATTTGGTCAGCAAATTGAA-3') and NAD4-R (5'-GCCTGCAAATGAATTAACA-3') were applied according to Yin *et al.*(2013) to produce 800 bp PCR products (Yin *et al.*, 2013). The PCR reaction mixtures consisted of 1x Ultra-Pure *Taq* PCR master mix (1 U of Ultra-Pure *Taq* polymerase, 2 mM $MgCl_2$ and 200 µM of each dNTPs) (Geneaid Biotech Ltd., Taiwan), 0.8 µM of each primer, and 2 µL of DNA template. The PCR cycle conditions were performed in a thermocycler (Biometra® T-gradient Thermoblock Thermal Cycler, Germany) with the initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min. After final extension at 72 °C for 7 min, the PCR products were cooled down to 20 °C.

The ITS-2 and *nd4* PCR products were determined on 1.5% agarose gel electrophoresis. The single DNA band was excised under UV-light and purified using the Genep Hlow™ Gel/PCR Kit (Geneaid Biotech Ltd., Taiwan). Then, the purified PCR products were sent to a DNA sequencing service which was performed in an ABI PRISM® 3730xl DNA sequencer (Biobasic Inc., Canada).

2.3 Data analysis

All 21 DNA sequences (231 bp for ITS-2 and 800 bp for *nd4*) were edited using MEGA 7.0 software (Kumar, Stecher, & Tamura, 2016) and aligned with sequences in the GenBank database at the website (http: / / www. ncbi. nlm. nih.gov/) using the BLASTn tool for the percentage similarity determination. Multiple sequence alignments were then analyzed by ClustalW and the pairwise sequence identity and nucleotide substitutions (transition and transversion) were determined with published sequences of *H. contortus* and *H. placei* from the GenBank database running in the BioEdit program (Hall, 1999). The phylogenetic tree was constructed using the UPGMA method for the ITS-2 analysis and the minimum-evolution methods for the *nd4* analysis in the MEGA 7.0 program. The evolutionary distances were computed using the Kimura 2-parameter method. The intra-population diversity parameters including nucleotide diversity, haplotypes diversity, average number of nucleotide differences, and DNA polymorphism was evaluated using DnaSP version 5.10.01 (Librado & Rozas, 2009).

3. Results and Discussion

3.1 Infestation of parasite nematodes in goats at Cha-am and species identification

The parasite nematodes were surveyed in 30 slaughtered goats from August to November 2015 at Cha-am District. All parasites were collected from the abomasum of the goats and identified according to species and sex under a stereo microscope. The prevalences of *Haemonchus* spp. and *Mecistocirrus digitatus* infestations were 86. 67% (26/30 goats) and 76.67% (23/30 goats), respectively. After *Haemonchus* spp. species identification, co-infection of *H. contortus* and *H. placei* was found in 24 goats. The prevalence of *H. contortus* was 80% (24/30 goats) while *H. placei* was 86.67% (26/30 goats). Most of the *H. contortus* worms in this area were female (94. 74%), whereas male was rarely found (5.26%). The females of the *H. contortus* were selected from 342 female worms and grouped into 20 samples to represent each individual goat for genetic variation analysis.

The parasite samples collected from the abomasum of goats found co-infection of *H. contortus* and *H. placei* more often than mono-infection. This infestation status was reported in small ruminants such as sheep, goats, and cattle that showed co-infection higher than mono-*H. contortus* and mono-*H. placei* infections in Tunisia (Akkari *et al.*, 2013). In contrast to the number of hybrid isolates (*H. contortus* x *H. placei*) in ruminants from Pakistan were less than the mono-infection (Hussain *et al.*, 2014).

3.2 Internal transcribed spacer-2 (ITS-2) sequence analysis

The 321 bp PCR products of ITS-2 sequences were sequenced from 20 samples from different goats. Twenty-four of 30 goats had *H. contortus* infestation but only 20 samples could be analyzed in Cha-am District (GenBank accession no. MF398432-MF398452). After sequence editing in the MEGA 7. 0 program, only the consensus length 231 bp of ITS-2 sequences were analyzed. The average GC and AT contents of the ITS-2 were 34.1% and 65.9%, respectively. The alignment of all 20 ITS-2 with the reference sequences (KP101363, KP101370, X78803, and EU084691) revealed that 15 polymorphic sites were 6 singleton variable sites and 9 parsimony informative sites. These substitution sites were 7 transitions (4:T<->C, 3:A<->G) at positions 52, 58, 74, 76, 187, and 208, and 8 transversions (5: A<->C, 1: G<->C, 2: A<->T) at positions 32, 45, 48, 59, 80, 93, 121, and 165. There were 9 genotypes of 20 ITS-2: genotype 1, 5 sequences, genotype 2, 7 sequences, and another one sequence of each genotype 3 to 9 (Table 1). The nucleotide diversity and genotype diversity were 0. 017 and 0. 832± 0. 063, respectively. The pairwise sequence identity among the 20 ITS-2 sequences revealed that the identities ranged from 93. 5 to 100% (98. 1% ± 1. 4) compared with four reference sequences for *H. contortus* (X78803, EU084691, KP101370, and KP101363) and *H. placei* (KF364626) which showed identity of 93. 5 to 99% (96.9%±1.2) (Table 2). Nucleotide alignment of all 20 ITS-2 sequences with the reference sequences in the GenBank database showed 96 to 99% (97.8% ± 0.9) similarity to *H. contortus* (KP101370, KP101381, KP101374, and KP090291) and 93 to 96% (95.6%±0.9) for *H. placei* (KF364625). These sequences were similar to Thai genotypes and classified in G8 genotype (KP101370), G19 (KP101381), and G12 (KP10 1374).

Table 1. Nucleotide polymorphic sites among 9 genotypes from 20 ITS-2 sequences of *H. contortus*in goats at Cha-am District, Phetchaburi Provice, Thailand.

Genotypes	Nucleotide variable positions of ITS-2 sequence														
	32	45	48	52	58	59	61	74	76	80	93	121	165	187	208
G1	A	T	C	T	T	T	G	T	C	C	A	A	A	G	G
G2	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A
G3	.	.	G	.	.	A	.	.	.	.	.	.	.	.	.
G4	.	A	G	C	C	A	.	C	.	A	.	.	.	A	A
G5	.	.	.	.	C	A	.	.	.	.	.	.	.	A	A
G6	.	.	.	.	.	A	.	.	.	.	.	.	.	A	A
G7	.	A	G	C	.	.	.	C	.	.	.	.	.	.	.
G8	.	A	G	.	.	A	.	.	.	A	.	.	.	.	.
G9	C	.	.	.	.	.	A	.	T	.	C	C	C	A	A

Note: A dot indicates an identical nucleotide sequence comparing with sequence G1.

Table 2. Pairwise identities (%) among 20 ITS-2 sequences of *H. contortus* representing 20 parasites in goats at Cha-am District, Phetchaburi Province, Thailand compared with the published ITS-2 sequences of *H. contortus* and *H. placei* from the GenBank database.

Sequence Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
HCI01																									
HCI02	99.00																								
HCI03	99.00	98.00																							
HCI04	99.00	100.00	98.00																						
HCI05	95.50	96.50	96.50	96.50																					
HCI06	99.00	100.00	98.00	100.00	96.50																				
HCI07	98.50	99.50	97.50	99.50	96.00	99.50																			
HCI08	99.00	98.00	100.00	98.00	96.50	98.00	97.50																		
HCI09	97.50	98.50	97.50	98.50	97.00	98.50	99.00	97.50																	
HCI10	98.50	99.50	98.50	99.50	97.00	99.50	99.00	98.50	99.00																
HCI11	98.00	97.00	98.00	97.00	97.50	97.00	96.50	98.00	95.50	96.50															
HCI12	98.00	97.00	99.00	97.00	97.50	97.00	96.50	99.00	96.50	97.50	98.00														
HCI13	98.50	99.50	97.50	99.50	96.00	99.50	100.00	97.50	99.00	99.00	96.50	96.50													
HCI14	99.00	100.00	98.00	100.00	96.50	100.00	99.50	98.00	98.50	99.50	97.00	97.00	99.50												
HCI15	99.50	98.50	98.50	98.50	95.00	98.50	99.00	98.50	98.00	98.00	97.50	97.50	99.00	98.50											
HCI16	99.50	98.50	98.50	98.50	95.00	98.50	99.00	98.50	98.00	98.00	97.50	97.50	99.00	98.50	100.00										
HCI17	96.00	97.00	95.00	97.00	93.50	97.00	96.50	95.00	95.50	96.50	94.00	94.00	96.50	97.00	95.50	95.50									
HCI19	99.00	100.00	98.00	100.00	96.50	100.00	99.50	98.00	98.50	99.50	97.00	97.00	99.50	100.00	98.50	98.50	97.00								
HCI20	99.50	98.50	98.50	98.50	95.00	98.50	99.00	98.50	98.00	98.00	97.50	97.50	99.00	98.50	100.00	100.00	95.50	98.50							
HCI21	100.00	99.00	99.00	99.00	95.50	99.00	98.50	99.00	97.50	98.50	98.00	98.00	98.50	99.00	99.50	99.50	96.00	99.00	99.50						
X78803-HC	99.00	98.00	98.00	98.00	94.50	98.00	97.50	98.00	96.50	97.50	97.00	97.00	97.50	98.00	98.50	98.50	95.00	98.00	98.50	99.00					
EU084691-HC	100.00	99.00	99.00	99.00	95.50	99.00	98.50	99.00	97.50	98.50	98.00	98.00	98.50	99.00	99.50	99.50	96.00	99.00	99.50	100.00	99.00				
KP101370-HCG8	100.00	99.00	99.00	99.00	95.50	99.00	98.50	99.00	97.50	98.50	98.00	98.00	98.50	99.00	99.50	99.50	96.00	99.00	99.50	100.00	99.00	100.00			
KP101363-HCG1	100.00	99.00	99.00	99.00	95.50	99.00	98.50	99.00	97.50	98.50	98.00	98.00	98.50	99.00	99.50	99.50	96.00	99.00	99.50	100.00	99.00	100.00	100.00		
KF364626-HP	98.00	97.00	97.00	97.00	93.50	97.00	96.50	97.00	95.50	96.50	96.00	96.00	96.50	97.00	97.50	97.50	94.00	97.00	97.50	98.00	99.00	98.00	98.00	98.00	

A phylogenetic tree of the 20 ITS-2 sequences was constructed using the UPGMA method (Kimura M., 1980) in the MEGA7 program and computed with two Thai *H. contortus* reference sequences (KP101363 and KP101370) and *H. placei* (KF364626) as the outgroup. The tree was divided into three main groups. The first group was 18 ITS-2 sequences from *H. contortus* in this study and two reference sequences (KP101363 and KP101370). The second group was two ITS-2 sequences number HCI17(MF398448) and HCI18(MF398449). The third group was *H. placei* (KF364626) which was separated to be the outgroup (Figure 1). For the specie identification, the ITS-2 analyses from 20 sequences were confirmed to specifically identity *H. contortus.* The number of ITS-2 genotype in this report was 9 genotypes while a previous report found 21 genotypes from 11 provinces (except Phetchaburi) in Thailand (Mangkit *et al.*, 2014). *Haemonchus* ITS-2 genotype was observed in several countries such as Malaysia and Yemen (6 genotypes) (Gharamah *et al.*, 2012), China (18 genotypes) (Yin *et al.*, 2013), and Pakistan (12 genotypes) (Hussain *et al.*, 2014). Interestingly, the number of polymorphic sites of ITS-2 in this study was 15 sites which was near the Pakistan isolates of 14 polymorphic loci and the positions 58 and 59 were also observed as nucleotide variations (Hussain *et al.*, 2014). The polymorphic sites in the ITS-2 sequence of *H. contortus* from previous reports varied in positions 10, 6, 14, and 3 in Tunisia (Akkari *et al.*, 2013), China, Pakistan, Malaysia, and Yemen. Recently in Thailand, Mangkit *et al.* (2014) reported 12 polymorphic sites and found the variation of nucleotide T<->A at position 59 which was the same as in this study, including the specie-specific sequence at positions 205 and 219 which were found only in *H. placei* (Mangkit *et al.*, 2014). The GC content in the ITS-2 sequence was 34.1% that was reported in some other studies: 36% from goats in Tunisia (Akkari *et al.*, 2013), 32.9% from goats in 11 provinces of Thailand (Mangkit *et al.*, 2014), and 33.4% from goats in Malaysia and Yemen (Gharamah *et al.*, 2012). The nucleotide diversity in this study was low (Pi = 0.017) which was similar to the ITS-2 sequence from *H. contortus* in goats at other regions of Thailand (0.007), whereas the high genotype diversity was observed in both reports (0.832±0.063 and 0.724±0.025) (Mangkit *et al.*, 2014). Many host-related factors have an effect on the high genotype diversity of parasites such as cross infection and circulation among heterologous hosts. Parasites are derived from different ruminant host species, the movement of animal hosts within a country or between countries, and the lack of anthelminthic selection pressure (Akkari *et al.*, 2013). Although, all parasites in this study were collected from goats in the small area at Cha-am District, the high genotype diversity was possibly caused by different origins of the individual goats in this area which did not come from goat farming. The pairwise sequence identity among the 20 ITS-2 sequences of *H. contortus* to the four published ITS-2 sequences of *H. contortus* in the GenBank database was also determined to average 98.1% which was similar to the pairwise sequence identity among 21 genotypes of *H. contortus* from other regions of Thailand (98.5%). In addition, the pairwise identity between *H. contortus* and *H. placei* (outgroup) showed the same results at 96.9% identity (Mangkit *et al.*, 2014). In the other previously published reports, sequence identity was also analyzed in Tunisia (98% homology) (Akkari *et al.*, 2013) and in China where identity ranged from 97.4 to 100% (Yin *et al.*, 2013). The phylogenetic analysis of all 20 ITS-2 sequences revealed 18 from 20 sequences grouped together with *H. contortus* genotype 1 (KP101363) and genotype 8 (KP101370) from other provinces in Thailand. This result suggested that the genetic relationship of *H. contortus* from goats in Cha-am District was closely related to *H. contortus* from different areas in Thailand.

3.3 Nicotine amide dehydrogenase subunit 4 (*nd4*) gene analysis

For the genetic diversity study, the total 621-bp length of 19 sequences from partial *nd4* (nicotine amide dehydrogenase subunit 4) gene was sequenced (GenBank accession no. MF380898-MF380916). This region was located at nucleotide positions 13220 to 13840 of the complete mitochondrial genome of *H. contortus* (GenBank

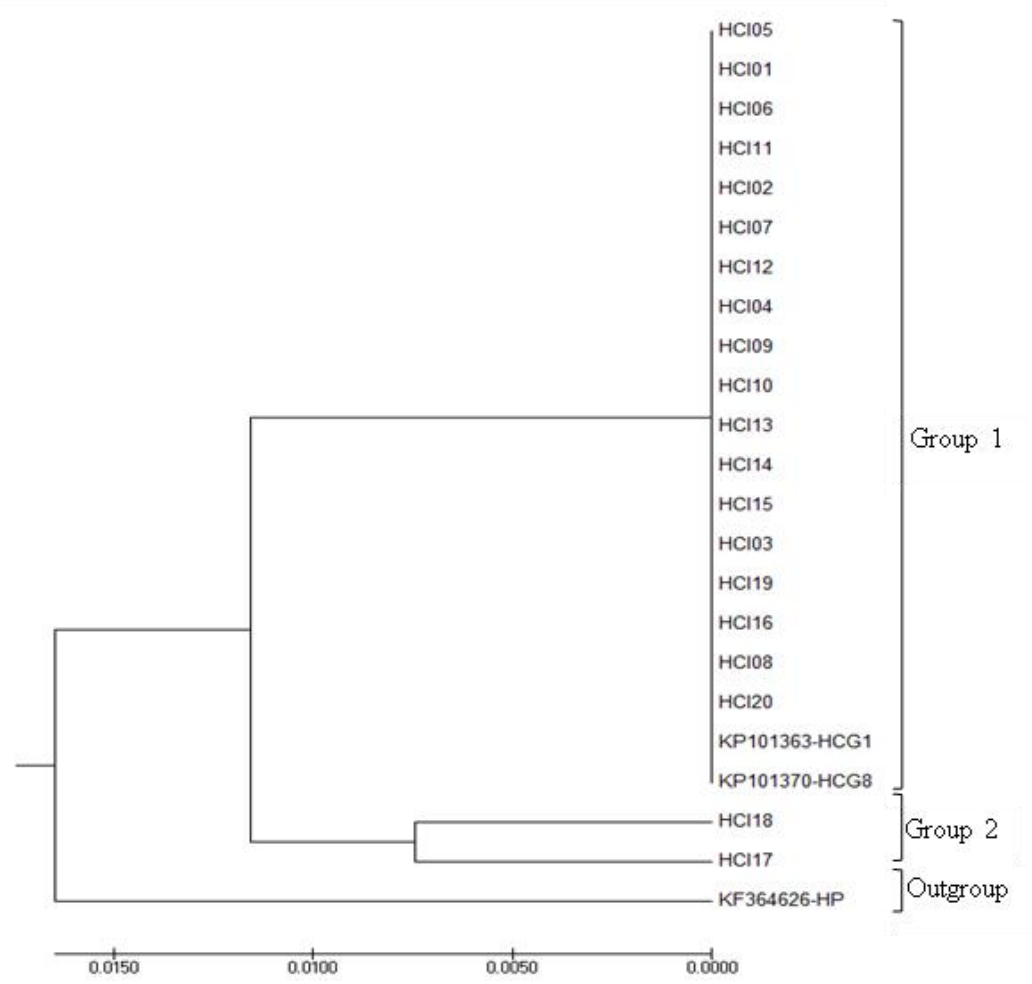

Figure 1. Phylogenetic tree constructed using the UPGMA method. The evolutionary distances were computed using the Kimura 2-parameter method in the MEGA7 program. The 20 ITS-2 sequences from *H. contortus* from Cha-am District, Phetchaburi Province, Thailand were compared with two *H. contortus* (KP101363 and KP101370) and *H. placei* (KF364626) as the outgroup.

accession no. EU346694). The number of polymorphic sites was 87 sites in which 41 were singleton variable sites and 46 were parsimony informative sites. The total number of mutations revealed 91 sites. The nucleotide diversity was determined to be 0.035 with the average number of nucleotide differences of 21.92. The number of haplotypes observed in *nd4* sequences found 19 haplotypes with an overall haplotype diversity of 1.00. The genetic diversity of *nd4* sequences in *H. contortus* was also observed with high diversity in Yemenese, Malaysian, and Pakistani isolates (Gharamah *et al.*, 2012; Hussain *et al.*, 2014; Yin *et al.*, 2013).

In order to genetically compare *H. contortus* from Cha-am District to other countries in the world, phylogenetic trees were constructed from all 19 *nd4* sequences and another six *nd4* sequences from USA (AF070736), Malaysia (HQ660353), Italy (AJ429793), China (KC429944), Yemen (HQ660278), and Pakistan (KJ724439). The *nd4* sequences from *H. placei* (AF070825) were used as the outgroup. All 26 *nd4* sequences were aligned and adjusted in the BioEdit program to produce a 225-bp length overlapped site. The phylogenetic tree analysis using the minimum evolution method was computed in the MEGA7 program. Most of the *nd4* sequences from this study (18 sequences) were grouped in the same clade of *nd4* sequences from Pakistan, China, and Italy, while only one *nd4* (N05) was grouped with Yemen (Figure 2).

The mtDNA was used as the molecular marker for evolutionary studies and population structure of the ruminant since this gene has a higher mutation rate of substitution than does nuclear DNA (Troell *et al.*, 2006). In this study, the partial *nd4* gene (621-bp) showed a nucleotide diversity of 0.035 which was related to previously published data for this gene in China (0.018-0.037), Italy (0.026-0.03), USA (0.024-0.03), Malaysia (0.032-0.044), and Yemen (0.021-0.036) (Blouin, Yowell, Courtney, & Dame, 1995; Cerutti *et al.*, 2010; Gharamah *et al.*, 2012; Yin *et al.*, 2013). Similarly, the haplotype diversity of all 19 haplotypes was 1.00 which corresponded to the isolates of Pakistan (0.0998), Malaysia (0.0993), and China (0.0996). Moreover, the high degree of diversity of the *nd4* sequence in *H. contortus* in different countries was also observed: 42 haplotypes from 50 individual *H. contortus* in the USA (Blouin, Yowell, Courtney, & Dame,

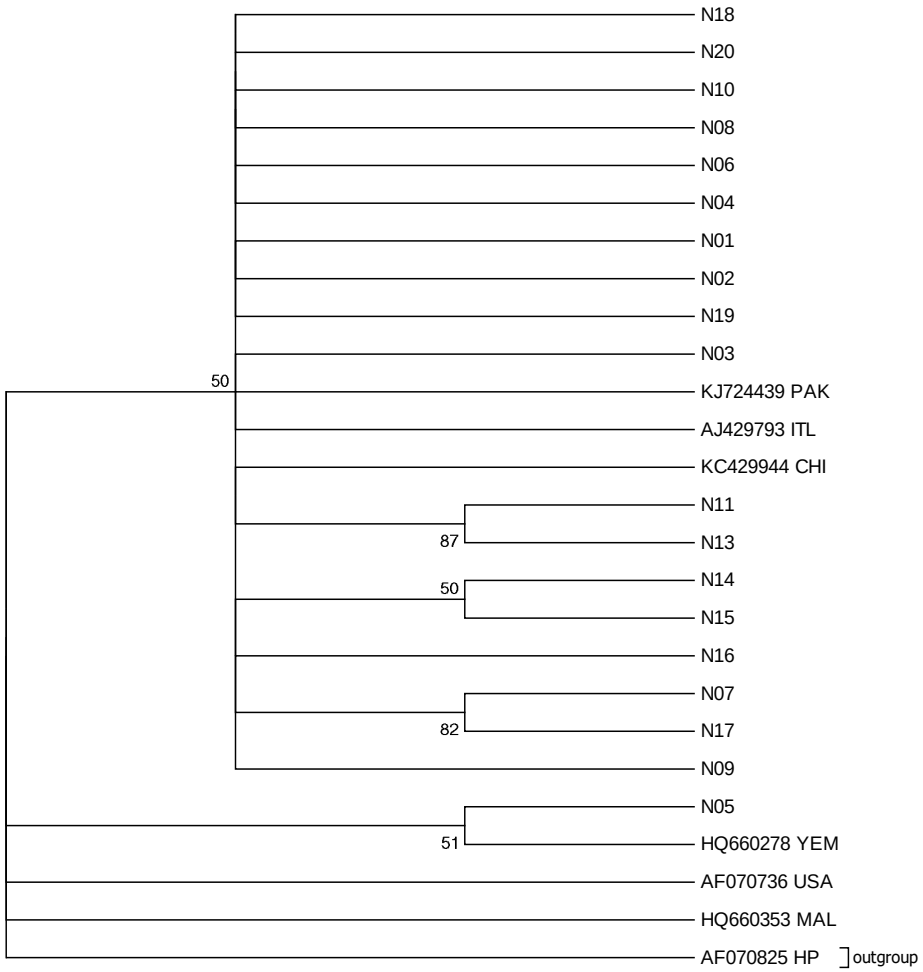

Figure 2. Phylogenetic tree constructed using the minimum evolution method in the MEGA7 program that shows the relationships among the 19 *nd4* sequences (225 bp overlapped regions) and another six *nd4*sequences from the GenBank database: USA (AF070736), Malaysia (HQ660353), Italy (AJ429793), China (KC429944), Yemen (HQ660278), and Pakistan (KJ724439). The *nd4* sequences from *H. placei* (AF070825) were used as the outgroup.

1997), 142 haplotypes from 152 individuals in China (Yin *et al.*, 2013), and 71 haplotypes from 81 haplotypes of *Haemonchus* parasites in Pakistan (Hussain *et al.*, 2014). In other nematodes from sheep using the *nd4* gene, 77 haplotypes were found among 85 individual sequences (Braisher, Gemmell, Grenfell, & Amos, 2004). A phylogenetic analysis of the *nd4* gene revealed all 19 haplotypes were grouped in the same clade of Pakistan, Italy, and China isolates (Figure 2). This finding might assume that *H. contortus* in Cha-am District possibly originated from a closely related species. Although, the data from this study came from the population within one province of Thailand, it can fill the gaps of information of the genetic variation in *H. contortus* and further assist in an anthelminthic program in the management of goats.

4. Conclusions

This study provided information on the genetic variation within *H. contortus* from slaughtered goats in Cha-am District, Phetchaburi Province, Thailand. The results revealed high genetic diversity which was 9 genotypes (ITS-2) and 19 haplotypes (*nd4*) among 20 parasites from different hosts. Most of the parasites showed a genetic relationship within the group and other parasites (genotype 1 and 8) from other places in Thailand. This observation might have implications for further studies in the parasite epidemiology and parasite control program in Thailand.

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